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Antibacterial Potential of *Phyllanthus niruri* L.

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The organic and aqueous leaf extracts of *Phyllanthus niruri* L. were studied against four bacterial pathogens. The antimicrobial susceptibility was screened using the disc diffusion method. The results showed that it was active against both Gram – positive bacteria (*Staphylococcus aureus)* and Gram – negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhi)* studied. The organic leaf extracts revealed potential antimicrobial activity. Whereas, the aqueous extract has no impact on the microbes studied. Out of the four organic leaf extracts studied the DMSO was highly effective against all the four bacteria, while the effect of acetone and methanol extract of leaf was moderate on pathogens and very low inhibition was shown by ethanolic extract. This study indicates that the *Phyllanthus niruri* dry leaf possesses a potential broad spectrum of antimicrobial activity.

Key words: *Phyllanthus niruri*, Antibacterial potential, Gram positive and negative bacteria.

Medicinal plants are essential natural resource which constitute one of the potential sources of new products and bioactive compounds for drug development (Gangwar *et al*., 2010). It is estimated that 60% of the world population and 80% of the population of developing countries rely on traditional medicines mostly plant drugs for their primary health care needs (Shrestha and Dhillon, 2003). Reports show that 70% of population of India is dependent on traditional plant based medicines (Gadgil and Rao, 1998).

The utilization of *Phyllanthus niruri* in traditional medicine has a long history. The *Phyllanthus niruri*, a herb of Euphorbiaceae family is indigenous to India. Medicinally it is known for its hepatoprotective activity, lipid lowering action, antidiabetic, antifungal, antiperiodic, diuretic, stomachic and demulcent action. It holds a reputed position in both Ayurvedic and Unani systems of medicine and has been used traditionally for curing jaundice, gonorrhea, frequent menstruation, diabetes, skinulcers, sores, swelling and itching (Meixa *et al*., 1995). The young shoots of the plants are administered in the form of an infusion for the treatment of chronic dysentery. The use of plant extracts and their phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits which are due to

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compounds synthesized in the secondary metabolism of the plant. As the demand for more and more drugs from plant sources is continuously increasing, there is a need to survey medicinal plants for their antimicrobial activity (Panda *et al*., 2009). Hence, the present research was taken up to evaluate the antibacterial potential of the both organic and aqueous leaf extracts of the plant.

MATERIAL AND METHODS

The materials for the present study were collected from the fields of Tiruchirappalli, Tamil Nadu, India. The plant was taxonomically identified with The Flora of Presidency of Madras (Gamble, 1957) and The Flora of Tamil Nadu Carnatic The Rapinat Herbarium, St. Joseph's College (Autonomous) Tiruchirappalli - 2. Tamil Nadu, India (Matthew, 1983). The aerial parts of the plant were thoroughly washed with clean water to remove earthy matter and spread on a clean surface for drying. The material was properly air dried for one week at room temperature of 30ºC. The dried parts were finely ground to a powder in an electric blender. The organic and aqueous extracts of plant samples were prepared by soaking 200 mg powder in 1ml of different aqueous and organic solvents.

Test organisms

The extracts of *P. niruri* were screened against a total of four pathogenic bacteria clinical isolates. The pathogenic bacterial strains used were obtained from Department of Clinical Microbiology, K.A.P.V. Government medical college, Tiruchirappalli, Tamil Nadu, India. The pathogenic strains used for testing the antibacterial activity of *P. niruri* include a Gram – positive bacterium (*Staphylococcus aureus*) and three Gram – negative bacterium (*Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*). All the microbial cultures were maintained on semi solid medium at 4°C with a subculture period of one week.

Standardization of inoculum

The organisms from the semisolid nutrient medium were inoculated into peptone water. After 6 hrs of inoculation, a loop full of peptone water with inoculum was streaked on Muller Hinton agar to check the purity. About 3-5 pure colonies of each organism were inoculated into normal saline and the turbidity was adjusted to the Mc Farlands scale (150×10^6 cfu/ml).

Preparation of Medium

Muller Hinton Agar was prepared in a conical flask, plugged with sterile cotton and autoclaved (121ºC) for 20min. Then the medium was allowed to equilibrate in a water bath to a constant temperature (50°C). The medium was poured into a sterile Petri plate and was left to solidify.

Preparation of discs of antibacterial agents

Discs of antibacterial agents were prepared from stock solution (1ml = 200mg). The concentrations used for the study were 100, 200, 400, 800, 1600, 3200 and 6400µg/ml. The above mentioned concentrations were prepared by loading the required micro liters on sterile what man paper disc of 6mm diameter. The discs were allowed to dry and were stored in air tight sterile containers.

Antibacterial testing

The plant extracts were tested for antibacterial activity using disc diffusion assay (Rasoanaiva & Ratsimamanga – Urverg, 1993). Four bacterial strains namely *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphyloccous aureus* were the micro organisms chosen for the experiment. The test organisms were inoculated with the help of a sterile cotton swab soaked in respective bacterial culture grown in peptone broth. The disc containing different concentrations of the plant part extract were placed over the solidified agar in such a way that there is no over lapping of zone of inhibition. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism inoculated in the Petri dishes were incubated in a thermostat at 37°C for 24 hours. The zone of inhibition produced by various concentrations of plant extracts on different organisms were measured and recorded by using a zone reader (Udayakumar & Hazeena Begum, 2002).

RESULTS AND DISCUSSION

The results of antibacterial potential were presented in Table 1. The results of the investigation showed that all the four selected bacterial strains in the present study were inhibited by different organic solvent leaf extracts of *Phyllanthus niruri* at different degrees.

Table 1. Antibacterial activity of *Phyllanthus niruri* leaf extract against some pathogenic bacteria

S. No.	Test Organism	AQ					AC					DMSO					ET					ME				
		100 µg	200 µg	400 µg	800 µg	1600 3200 µg µg	100 µg	200 µg	400 µg	800 µg	1600 3200 µg µg	100 µg	200 µg	400 µg	800 µg	1600 3200 µg µg	100 µg	200 µg	400 µg	800 µg	1600 3200 µg µg	100 µg	200 µg	400 µg	800 µg	1600 3200 µg µg
1	*E. coli*	-	-	-	-	- -	-	-	-	-	+ ++	++	+++	++	++	+++ +++	-	+	+	++	- -	-	-	+	+	++ ++
2	*K. pneumoniae*	-	-	-	-	- -	-	+	+	+	++ ++	++	+++	++	++	+++ +++	-	+	++	++	++ ++	+	++	++	++	++ ++
3	*S. typhi*	-	-	-	-	- -	-	+	+	+	++ ++	++	+++	++	++	+++ +++	-	+	+	+	++ -	-	-	+	++	++ ++
4	*S. aureus*	-	-	-	-	- -	-	-	-	++	++ ++	++	+++	++	++	+++ +++	-	+	++	++	+ +	+	++	++	++	++ ++

- = No inhibition of growth;
+ = Scanty inhibition of growth (7-11mm);
++ = Moderate inhibition of growth (11 – 15mm);
+++ = Heavy inhibition of growth (>15mm);
AQ = Aqueous; AC = Acetone; DMSO = Dimethyl sulphoxide; ET = Ethanol,
ME = Methonal.

Out of the four organic solvents selected for extraction of leaves of *Phyllanthus niruri*, the DMSO extract of all selected concentrations (100 - 6400µg) showed significant growth inhibition. However, heavy growth inhibition (>15mm) of *E. coli* was recorded in DMSO leaf extract. At low concentrations (100 - 400 µg), the growth inhibition was moderate in (11 – 15mm) *K. pneumoniae* and *S. typhi*. The growth of the same bacterial strains were very effectively inhibited above 800 µg (>15mm). On the contrary, the DMSO leaf extract was able to cause growth reduction of *S. aureus* to only a moderate level at all concentrations (11 – 15mm).

The response of bacterial strains to acetone extract of leaf was slightly different. The highest zone of inhibition was recorded against *S. typhi* and *S. aureus* at a high concentrations of 1600 to 6400 µg. The maximum growth inhibition in *E. coli* and *K. pneumoniae* was observed only at 6400 µg. At 1600 and 3200µg the inhibition zone was measured between 11-15mm in diameter and it was a moderate zone. Below that concentration the effect was comparatively low.

Methanol extract of *Phyllanthus niruri* leaf revealed potential growth inhibition (>15mm) of *S. aureus* from 800µg onwards. Moderate growth inhibition in *K. pneumoniae* was noted between 100 to 1600 µg. At 3200 and 6400 µg concentrations, heavy growth inhibition was recorded. The methanol extracts of leaf showed potential growth inhibition of *E. coli* and *S. typhi* at 6400µg. Whereas, moderate inhibition was noted at 1600 and 3200µg. The inhibitory activity due to methanol extract of *Phyllanthus niruri* of our results is in agreement with the report of Thomas *et al*. (1999). In their study they proved the inhibitory effect of *Phyllanthus niruri* against *E. coli* and *S. aureus.*

A different trend of results was obtained for ethanol extracts of *Phyllanthus niruri* leaves. The effect of this solvent extract was in generally less when compared to other solvents used in the study. Heavy growth inhibition of *E. coli*, *S. typhi* and *S. aureus* was recorded only at 6400µg. Below that concentration the antibacterial activity was not very evident.

Aqueous leaf extracts did not show any antibacterial activity against the selected organisms. Similar to our results, aqueous leaf extracts of *Tinospora cordifolia* showed no

inhibition against the growth of *S. typhi* and *S. aureus*. However, there was less inhibition due to aqueous leaf extracts of *T. cordifolia* on *E. coli* (Jeyachandran and Anand, 2005). In the present study, aqueous extracts of *Phyllanthus niruri* showed negative results against all the 4 selected bacterial strains. On the contrary to our observation, a positive effect of aqueous extract from bark of *Azadirachta indica* on *S. aureus* was reported (Metta Ongsakul., *et al* 2009). In their observation, aqueous bark extract at 165µg and above showed widest average inhibition zone which indicated the most antibacterial effect against *S. aureus.*

The results of our investigation showed that the different solvent extracts of *Phyllanthus niruri* leaves possess appreciable antibacterial activity against all the four bacterial strains. Our results find supportive evidence from the studies of Uchechi *et al*. (2006) that the ethanol extract of *Phyllanthus niruri* showed remarkable antibacterial activity against *E. coli, S. typhi* and *S. aureus*.

CONCLUSIONS

The results obtained from our studies justify the use of *Phyllanthus niruri* in traditional medicine for the treatment of diseases. An interesting point to note is that the organic solvent dimethyl sulphoxide extracts of *P. niruri* showed remarkable antibacterial potential against *Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Staphylococcus aureus*. Hence, it may be recommended that these plants could be used in the treatment of human diseases caused by the above mentioned organisms.

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